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PATENT

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Date

Millicent A. Scarlett

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Martin A. Cheever and Mary L. Disis
Application No. : 09/088,951
Filed : June 2, 1998
For : METHODS AND COMPOSITIONS TO GENERATE
IMMUNITY IN HUMANS AGAINST SELF TUMOR
ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS
FOREIGN PROTEINS
Examiner : Karen A. Canella, Ph.D.
Art Unit : 1642
Docket No. : 920010.535

Commissioner for Patents
Washington, DC 20231

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DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner:

We, Martin A. Cheever and Mary L. Disis, hereby declare as follows:

1. We are co-inventors of the subject matter described in the above-identified patent application (hereinafter referred to as "subject application").

2. All of the work described within this Declaration was performed in the United States, by ourselves or on our behalf and under our direction.

3. We have reviewed our laboratory records, including the Exhibit submitted herewith, and readily conclude that methods, as claimed in the subject application, were conceived prior to October 1996. Further, due diligence was exercised from this time period until the invention was either actually reduced to practice, or until the filing of the subject application.

4. Prior to October 1996, we conceived the invention described in the subject application, i.e., that an immune response could be elicited or enhanced against self tumor antigens by immunization with homologous foreign proteins or portions thereof. In one embodiment of our invention, the self tumor antigen is prostatic acid phosphatase ("PAP"). Experiments from our laboratory confirmed our inventive concept by showing that immunization of rats with human PAP induced an immune response to rat PAP, whereas immunization of rats with rat PAP did not induce an immune response.

5. The following Exhibit (annexed hereto) represents a photocopy of laboratory notebook pages and a Western blot (all from prior to October 1996) kept in the regular course of business at the University of Washington. The dates have been removed from the copies submitted herewith. It our understanding, based on discussion with applicants' representatives, that this is a permissible Patent Office practice.

6. The Exhibit, which is a photocopy of several pages from laboratory notebooks and a Western blot (all from our laboratory), discloses the immunization of Fischer rats ("K") and Lewis rats ("CH") with PAP, e.g., rat PAP ("rPAP") or human PAP ("hPAP"). The Western blot shows that immunization of rats with hPAP elicited antibodies to both human and rat PAP.

7. Included with the Exhibit is a compilation of data from additional experiments (also all prior to October 1996) from our laboratory. The data validates that rat PAP did not elicit an immune response in rats, but immunization of rats with human PAP induced an immune response to both rat and human PAP. Moreover, following immunization of rats with human PAP, the immunized rats could now be boosted with rat PAP.

8. In summary, upon review of our laboratory records, of which the above-cited pages are representative, we have concluded that, at least prior to October 1996, we had conceived of the methods as described and claimed within the subject application. Furthermore, our conception of the invention led to further research, diligently undertaken, resulting in an actual reduction to practice or in the filing of the subject application.

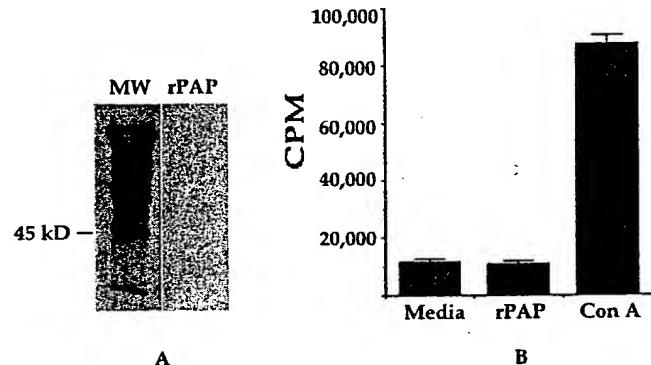
9. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Martin A. Cheever
Martin A. Cheever

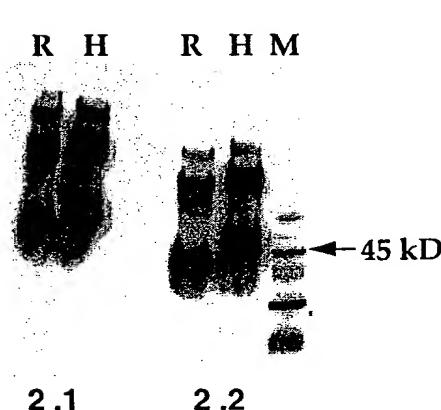
Dated this 5/5 day of April, 2002

Mary L. D'isis
Mary L. D'isis

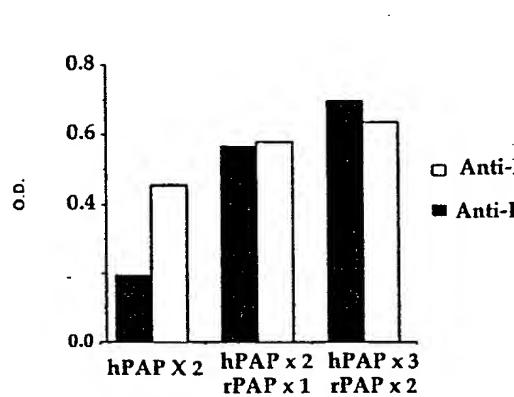
Dated this 14 day of April, 2002



No immunity to rat PAP was induced by immunization with whole rat PAP. Female Lewis rat were immunized with recombinant rat PAP (100 µg) admixed with CFA and boosted twice with rat PAP plus IFA at three-week intervals. The antibody response against rat PAP (rPAP) was determined by western blot analysis (A) as described in Fig. 1. The T cell response was determined by a standard proliferation assay (B). Histopaque-purified splenic mononuclear cells (5×10^5 /ml) were incubated with either media, rat PAP (200 µg/ml), or Con-A (5 µg/ml) for 96 hours. Tritiated thymidine (1 uCi/well) was added to the culture for the last 8 hours. The thymidine uptake by T cells was determined by liquid scintillation counting (cpm).



IgG antibody responses to rat PAP could be induced by sequential immunization with hPAP and rPAP. Female Lewis rats were immunized with human PAP (100 µg) plus CFA. They were boosted at three-week intervals with IFA plus human PAP (100 µg) and with IFA plus rat PAP (100 µg). Antibody responses to rat PAP or human PAP were determined by western blot analysis. Rat PAP (R) and human PAP (H) were run on a 10-15 % SDS-PAGE gel under reducing conditions. The blot was incubated with immune sera from two representative animals (2.1 and 2.2), followed by HRP-labeled goat anti-rat IgG antibody. The blot was developed with the ECL detection system. The molecular weight markers (M) are shown on the right.



IgG antibody responses to rat PAP after immunization with human PAP can be significantly boosted by subsequent injections with rat PAP. Female Lewis rats were immunized with human PAP (100 µg) plus CFA. They were boosted sequentially at 3-week intervals with IFA plus human and rat PAP (hPAP x2, rPAP x1); or IFA plus human and rat PAP (hPAP x3, rPAP x2). Sera were obtained two weeks after each boosting. The amount of antibody to rat PAP (solid bars) or human PAP (open bars) in the immune sera was determined by an ELISA assay with 96-well plates pre-coated with either rat PAP or human PAP, respectively. The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined.

Rk.C.

① ELISA[△] (Bengal r. -) 100%
100 µg 1:1200 1:800
1:1600 40%
1:2000 1:1000 57%

② CHIV 1 Rd ♀ Re Frank
 1 Rd ♂ Imm

④ Plates ⇒

③ Purification

Wednesday

Bent
PDSF

$K_1 \& K_{11}$

K_{12}

(\checkmark cells)

} cells

RV \rightarrow v Ah staken

2. Cut Rat &
 3. Sip in 0.5% T.T.C. / ARS
 + 1 ml of Bradford reagent
 ≈ 100 μl of sample + H₂O

981 963 971.9
 Mouse 2x 4x RV
 14.2

+ 1 ml of H₂O or Blank

Blank 0.00 595λ

Mouse 2x 0.257

→ 1.95 mg/ml

Mouse 4x 0.474

→ 1.8 mg/ml } 1.9 mg/ml

Rat 1x 0.447

→ 6.8 mg/ml }

2x 0.679

→ 1. }

0.25x 115

→ 8.8 mg/ml } Bnglm

• 0.66 = 1 μg

28 4nd ml

Ctrl Sec

Pre Inn

CH II

Sera

&
Book

CH II

Tues

Grants

Handbook

in
APL

CH III

Fri

Semi-Is

IP CH II

+ CH III

De

Salinity

Ag

ICL-2

→ Spleens

B

* Riss K Ne

100% buffer

CTRL

P39

Human

G

G

Nicely B Kent

Thursday

KII G
Spleen

* Book on Tues
CH II & CH III

~~KV~~

Serv & Saw-f-

? P45 (2) G8
? Bacon (4) OR
? & Freeze every 1h-

X

KZ

Out by

}

Ctr
 Htr
 P3S

San Tcell Adapt.
 Raung

P45
 P43

P45 A 640 1/3 3 flanks
 | (B) 140 1/3 3 flanks
 |

Ctr
 Htr
 P3S

San +
 Boost

San +
 Boost

Sanfe on

Ctr 45 San
 Sanfe Sanfe Freeze spleen

Risk will run individuals

Improve ~



(1)	→ C722	x 2	1
(2)	→ Human PTA	x 4	2 →
(3)	→ 39	x 6	2
(4)	→ 45	x 6	2
(5)	→ 43	x 2	2

1st 2mm

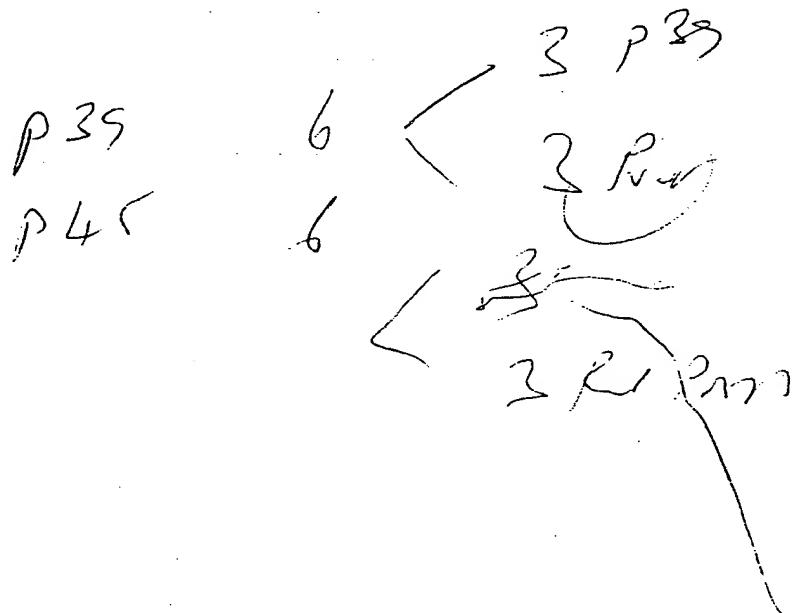
All ~~so~~

2nd 2mm

seen on

3rd 2mm

Can Weisens



from

Peyviller

750/ug each

Bovine PAP 400μg

KV

- ① CTRL → 2 1st Imm.
- ② Bovine PAP → 4 ~~1st~~
- ③ p39 → 6 ^{Boat} pSS, other pgs
- ④ p45 → 6 ~~p45~~ 62,727
- ⑤ p43 → 2 ~~1st~~

750

* Sera

+ Boat

* Do Sera from a Western

3rd Imm.

~~200 μg each~~ ~~from~~ ~~on~~
 Base 10⁰ Rev 10⁰ Human 10⁰
 10⁻¹ Rev 10⁻¹ Human 10⁻¹

~~KIV~~

~~2nd group~~

- ① → CTL
- ② → Bovine thal
- ③ → Bovine
- ④ → Human + Rev
- ⑤ → Human

1 Rev/group

remains

1st Imm →
2nd Imm →

} 1 Rev/group sacrificed

3rd Imm →

4th Imm →

70⁰⁰ → 4 cells

* ~~Sacrifice OTHER ONE =~~
 Imm over + latest S.D. or

Imm
and

* ✓ T cell results (single T.V.L.)

(cont)

R.v 2

①	✓	✓	✓	✓
②	✓	✓	✓	✓
③	✓	✓	✓	✓
④	✓	/	✓	✓
⑤	✓	✓	✓	✓
	10 cells		20 cells	

KIII

2 vials
4 + 1 sole
groups

✓

- ① → GM-CSF ✓
- ② → Bovine PAF + GM ✓
- ③ → Bovine PAF
- ④ → Galf + Rat PAF
- ⑤ → Rat PAF only (4 mm thick)

To do

* ✓ T cell results

* All rats sacrificed on

* Watering

9 gels



~~Pep. des~~

~~Zym~~

Pep. des 55 ✓
43 ✓
44 ✓
45 ✓
Protein 400ug total

(2)

Recd 1st Imm & IFA accidentally
2/group en

Sgroups

GRL

p39 + rPAP

p43

p44

p45 + rPAP

2 s.vcs QC & 2 s.vcs Zym

1st Imm
2nd Imm

Pep. des + Protein [400ug total]

(To Do)

* Immunize /

- Western blot

✓ Sera collect-

SERA on

KI

6 Groups



1+L/group

4th Imm

① → PBS/LFA / LFA → 4 Imm all live

② → p39 + 100ng/ml PAF

③ → p43

④ → p44

⑤ → p45
+ 100ng/ml PAF

⑥ → ~~PAK~~ PAF → Shaded & X3

All rats \bar{x} Group 6 → SACRIFICE -

To do

* ✓ Tcell results

* ~~Westerns~~ OR1 → 6 [6 gel]

HMW

HMW

Human

Homogenate

Ref

-

LMW

1.1

1.1

HMW

Human

Homogenate

Ref

-

LMW

2.1 & 2.2

5 sec